

1     **Claims**

2

3     1. A method of determining the presence of a  
4       toxicant in a test sample, comprising the steps  
5       of;

- 6           • exposing a eukaryote that has been  
7           transformed with a light emitting  $\text{Ca}^{2+}$   
8           regulated photoprotein gene to a test sample  
9           • measuring the light produced by the  
10          transformed cell/organism  
11          • determining whether the amount of light is  
12          above or below a defined threshold at the  
13          time of exposure.

14

15     2. A method as in Claim 1 wherein the eukaryote is a  
16       fungi.

17

18     3. A method as in Claim 2 wherein the fungi is a  
19       filamentous fungi.

20

21     4. A method as in Claims 2 or 3 wherein the fungi is  
22       of the *Aspergillus* species.

23

24     5. A method as in Claim 1 wherein the eukaryote is a  
25       mammalian cell.

26

27     6. A method as in Claim 1 wherein the eukaryote is a  
28       plant cell.

29

30     7. A method as in any of the previous Claims wherein  
31       the test sample comprises a toxicant.

32

1 8. A method as in any of the previous Claims wherein  
2 the light emitting  $\text{Ca}^{2+}$  regulated photoprotein  
3 gene is a recombinant gene.  
4

5 9. A method as in any of the previous Claims wherein  
6 the light emitting  $\text{Ca}^{2+}$  regulated photoprotein  
7 gene is selected from the group comprising;

- 8 • aequorin gene
- 9 • halistaurin (mitrocomin) gene
- 10 • phialidin (clytin) gene
- 11 • obelin gene
- 12 • mnemiopsin gene
- 13 • berovin gene

14

15 10. A method as in any of the previous Claims  
16 wherein the light emitting  $\text{Ca}^{2+}$  regulated  
17 photoprotein gene may be a functional homologue  
18 of a gene selected from the group comprising;

- 19 • aequorin gene
- 20 • halistaurin (mitrocomin) gene
- 21 • phialidin (clytin) gene
- 22 • obelin gene
- 23 • mnemiopsin gene
- 24 • berovin gene

25

26 11. A method as in any of the previous Claims  
27 wherein the light emitting  $\text{Ca}^{2+}$  regulated  
28 photoprotein gene is an aequorin gene.  
29

- 1 12. A method as in any of the previous Claims  
2 wherein the light emitting  $\text{Ca}^{2+}$  regulated  
3 photoprotein gene is a recombinant aequorin gene.  
4
- 5 13. A method as in any of the previous Claims  
6 wherein the light that is measured is in the form  
7 of luminescence.  
8
- 9 14. A method as in any of the previous Claims  
10 wherein the test sample is added in advance of  
11 the application of a stimulus to the test sample.  
12
- 13 15. A method as in Claim 14 wherein the stimulus is  
14 at least one or more from the group comprising;  
15 mechanical perturbation, hypo-osmotic shock,  
16 change in external calcium chloride  
17 concentration, temperature shock and pH shock.  
18
- 19 16. A method as in Claims 14 and 15 wherein the  
20 test sample is added 1 minute to 1 hour prior to  
21 the application of the stimulus.  
22
- 23 17. A method as in Claims 14 to 16 wherein the test  
24 sample is added 5 minutes prior to the  
25 application of the stimulus.  
26
- 27 18. A method as in Claims 14 to 16 wherein the test  
28 sample is added 30 minutes prior to the  
29 application of the stimulus.  
30

- 1     19. A method of determining the presence of a  
2       toxicant in a test sample, comprising the steps  
3       of;  
4       • exposing a eukaryote that has been  
5       transformed with a light emitting  $\text{Ca}^{2+}$   
6       regulated photoprotein gene to a test sample  
7       • measuring the light produced by the  
8       transformed cell/organism  
9       • determining whether the amount of light is  
10      above a defined threshold at a specified  
11      time after the time of exposure.  
12
- 13    20. A method as in Claim 19 which comprises the  
14      step of determining whether the amount of light  
15      is below a defined threshold.  
16
- 17    21. A method as in Claims 19 and 20 wherein the  
18      specified time after the time of exposure is 11  
19      minutes.  
20
- 21    22. A method as in Claims 19 to 21 wherein the  
22      eukaryote is a fungi.  
23
- 24    23. A method as in Claim 22 wherein the fungi is a  
25      filamentous fungi.  
26
- 27    24. A method as in Claims 22 to 23 wherein the  
28      fungi is of the *Aspergillus* species.  
29
- 30    25. A method as in Claims 19 to 21 wherein the  
31      eukaryote is a mammalian cell.  
32

- 1     26. A method as in Claims 19 to 21 wherein the  
2         eukaryote is a plant cell.  
3
- 4     27. A method as in Claims 19 to 26 wherein the test  
5         sample comprises a toxicant.  
6
- 7     28. A method as in Claims 19 to 27 wherein the  
8         light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is  
9         a recombinant gene.  
10
- 11    29. A method as in Claims 19 to 28 wherein the  
12         light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is  
13         selected from the group comprising;  
14         • aequorin gene  
15         • halistaurin (mitrocomin) gene  
16         • phialidin (clytin) gene  
17         • obelin gene  
18         • mnemiopsin gene  
19         • berovin gene  
20
- 21    30. A method as in Claims 19 to 29 wherein the  
22         light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene  
23         may be a functional homologue of a gene selected  
24         from the group comprising;  
25         • aequorin gene  
26         • halistaurin (mitrocomin) gene  
27         • phialidin (clytin) gene  
28         • obelin gene  
29         • mnemiopsin gene  
30         • berovin gene  
31

- 1     31. A method as in Claims 19 to 30 wherein the  
2         light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is  
3         an aequorin gene.  
4
- 5     32. A method as in Claims 31 wherein the light  
6         emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is a  
7         recombinant aequorin gene.  
8
- 9     33. A method as in Claims 19 to 32 wherein the  
10        light that is measured is in the form of  
11        luminescence.  
12
- 13    34. A method as in Claims 19 to 33 wherein the test  
14        sample is added in advance of the application of  
15        a stimulus to the test sample.  
16
- 17    35. A method as in Claim 34 wherein the stimulus is  
18        at least one or more from the group comprising;  
19        mechanical perturbation, hypo-osmotic shock,  
20        change in external calcium chloride  
21        concentration, temperature shock and pH shock.  
22
- 23    36. A method as in Claims 34 to 35 wherein the test  
24        sample is added 1 minute to 1 hour prior to the  
25        application of the stimulus.  
26
- 27    37. A method as in Claims 34 to 36 wherein the test  
28        sample is added 5 minutes prior to the  
29        application of the stimulus.  
30

1 38. A method as in Claims 34 to 36 wherein the test  
2 sample is added 30 minutes prior to the  
3 application of the stimulus.  
4

5 39. A method of determining the presence of a  
6 toxicant in a test sample, comprising the steps  
7 of;

- 8 • exposing a eukaryote that has been  
9 transformed with a light emitting  $\text{Ca}^{2+}$   
10 regulated photoprotein gene to a test sample
- 11 • measuring the light produced by the  
12 transformed cell/organism
- 13 • and comparing the light measurement data  
14 with a bank of known toxicity reference  
15 data.  
16

17 40. A method as in Claim 39 wherein the method  
18 comprises the step of determining whether the  
19 amount of light is below a defined threshold.  
20

21 41. A method as in Claims 39 to 40 wherein the  
22 specified time after the time of exposure is 11  
23 minutes.  
24

25 42. A method as in Claims 39 to 40 wherein the  
26 eukaryote is a fungi.  
27

28 43. A method as in Claim 42 wherein the fungi is a  
29 filamentous fungi.  
30

31 44. A method as in Claims 42 to 43 wherein the  
32 fungi is of the *Aspergillus* species.

- 1  
2 45. A method as in Claims 39 to 41 wherein the  
3 eukaryote is a mammalian cell.  
4  
5 46. A method as in Claims 39 to 41 wherein the  
6 eukaryote is a plant cell.  
7  
8 47. A method as in Claims 39 to 46 wherein the test  
9 sample comprises a toxicant.  
10  
11 48. A method as in Claims 39 to 47 wherein the  
12 light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is  
13 a recombinant gene.  
14  
15 49. A method as in Claims 39 to 48 wherein the  
16 light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is  
17 selected from the group comprising;  
18     • aequorin gene  
19     • halistaurin (mitrocomin) gene  
20     • phialidin (clytin) gene  
21     • obelin gene  
22     • mnemiopsin gene  
23     • berovin gene  
24  
25 50. A method as in Claims 39 to 49 wherein, the  
26 light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene  
27 may be a functional homologue of a gene selected  
28 from the group comprising;  
29     • aequorin gene  
30     • halistaurin (mitrocomin) gene  
31     • phialidin (clytin) gene



- 1           • obelin gene
- 2           • mnemiopsin gene
- 3           • berovin gene

4

5       51. A method as in Claims 39 to 50 wherein the  
6       light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is  
7       an aequorin gene.

8

9       52. A method as in Claims 39 to 51 wherein the  
10       light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is  
11       a recombinant aequorin gene.

12

13       53. A method as in Claims 39 to 52 wherein the  
14       light that is measured is in the form of  
15       luminescence.

16

17       54. A method as in Claims 39 to 53 wherein the test  
18       sample is added in advance of the application of  
19       a stimulus to the test sample.

20

21       55. A method as in Claim 54 wherein the stimulus is  
22       at least one or more from the group comprising;  
23       mechanical perturbation, hypo-osmotic shock,  
24       change in external calcium chloride  
25       concentration, temperature shock and pH shock.

26

27       56. A method as in Claims 54 to 55 wherein the test  
28       sample is added 1 minute to 1 hour prior to the  
29       application of the stimulus.

30

1 57. A method as in Claims 54 to 56 wherein the test  
2 sample is added 5 minutes prior to the  
3 application of the stimulus.

4

5 58. A method as in Claims 54 to 55 wherein the test  
6 sample is added 30 minutes prior to the  
7 application of the stimulus.

8

9 59. A method as in Claims 39 to 58 wherein the  
10 method is used to determine the amount of  
11 toxicant in the sample.

12

13 60. A method as in Claims 39 to 59 wherein the  
14 method is used to identify the toxicant in the  
15 sample.

16

17 61. A method of determining the presence of a  
18 toxicant in a test sample, comprising the steps  
19 of;

- 20 • exposing a eukaryote that has been  
21 transformed with a light emitting  $\text{Ca}^{2+}$   
22 regulated photoprotein gene to a test sample
- 23 • measuring the light produced by the  
24 transformed cell/organism
- 25 • converting the light data into a cytosolic  
26 free calcium ion concentration trace,
- 27 • and comparing at least one parameter of the  
28 cytosolic free calcium ion concentration  
29 trace with a bank of known toxicity  
30 reference data.

31

- 1     62. A method as in Claim 61 wherein the method  
2         comprises the step of determining whether the  
3         amount of light is below a defined threshold.  
4
- 5     63. A method as in Claims 61 to 62 wherein the  
6         eukaryote is a fungi.  
7
- 8     64. A method as in Claim 63 wherein the fungi is a  
9         filamentous fungi.  
10
- 11    65. A method as in Claims 63 to 64 wherein the  
12         fungi is of the *Aspergillus* species.  
13
- 14    66. A method as in Claims 61 to 62 wherein the  
15         eukaryote is a mammalian cell.  
16
- 17    67. A method as in Claims 61 to 62 wherein the  
18         eukaryote is a plant cell.  
19
- 20    68. A method as in Claims 61 to 67 wherein the test  
21         sample comprises a toxicant.  
22
- 23    69. A method as in Claims 61 to 68 wherein the  
24         light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is  
25         a recombinant gene.  
26
- 27    70. A method as in Claims 61 to 69 wherein the  
28         light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is  
29         selected from the group comprising;  
30             • aequorin gene  
31             • halistaurin (mitrocomin) gene  
32             • phialidin (clytin) gene

- 1           • obelin gene
- 2           • mnemiopsin gene
- 3           • berovin gene

4

5       71. A method as in Claims 61 to 70 wherein the  
6       light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene  
7       may be a functional homologue of a gene selected  
8       from the group comprising;

- 9           • aequorin gene
- 10          • halistaurin (mitrocomin) gene
- 11          • phialidin (clytin) gene
- 12          • obelin gene
- 13          • mnemiopsin gene
- 14          • berovin gene

15

16       72. A method as in Claims 61 to 71 wherein the  
17       light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is  
18       an aequorin gene.

19

20       73. A method as in Claims 61 to 72 wherein the  
21       light emitting  $\text{Ca}^{2+}$  regulated photoprotein gene is  
22       a recombinant aequorin gene.

23

24       74. A method as in Claims 61 to 73 wherein the  
25       light that is measured is in the form of  
26       luminescence.

27

28       75. A method as in Claims 61 to 74 wherein the test  
29       sample is added in advance of the application of  
30       a stimulus to the test sample.

31

- 1     76. A method as in Claim 75 wherein the stimulus is  
2         at least one or more from the group comprising;  
3         mechanical perturbation, hypo-osmotic shock,  
4         change in external calcium chloride  
5         concentration, temperature shock and pH shock.  
6
- 7     77. A method as in Claims 75 to 76 wherein the test  
8         sample is added 1 minute to 1 hour prior to the  
9         application of the stimulus.  
10
- 11    78. A method as in Claims 75 to 77 wherein the test  
12         sample is added 5 minutes prior to the  
13         application of the stimulus.  
14
- 15    79. A method as in Claims 75 to 77 wherein the test  
16         sample is added 30 minutes prior to the  
17         application of the stimulus.  
18
- 19    80. A method as in Claims 61 to 79 wherein light is  
20         measured for between 1 minute and 5 hours  
21         following the application of the stimulus.  
22
- 23    81. A method as in Claims 61 to 79 wherein light is  
24         measured for between 1 minute and 96 hours  
25         following the application of the stimulus.  
26
- 27    82. A method as in Claims 61 to 79 wherein light is  
28         measured for 5 minutes following the application  
29         of the stimulus.  
30
- 31    83. A method as in Claims 61 to 82 wherein the  
32         cytosolic free calcium ion trace is a plot of the

1       cytosolic free calcium ion concentration against  
2       time.

3

4       84. A method as in Claims 61 to 83 wherein the  
5       parameter is at least one or more selected from  
6       the group comprising;

- 7           • lag time
- 8           • rise time
- 9           • absolute amplitude
- 10          • relative amplitude
- 11          • Length of transient at 20%, 50% and 80% of
- 12           maximum amplitude
- 13          • number of cytosolic free calcium ion
- 14           concentration increases
- 15          • percentage increase in final cytosolic free
- 16           calcium ion concentration resting level
- 17          • percentage increase in recovery time
- 18          • percentage increase in pre-stimulating
- 19           cytosolic free calcium ion concentration
- 20           resting level
- 21          • total concentration of calcium

22

23       85. A method as in Claims 61 to 84 wherein the  
24       method is used to determine the amount of  
25       toxicant in the sample.

26

27       86. A method as in Claims 61 to 85 wherein the  
28       method is used to identify the toxicant in the  
29       sample.

30